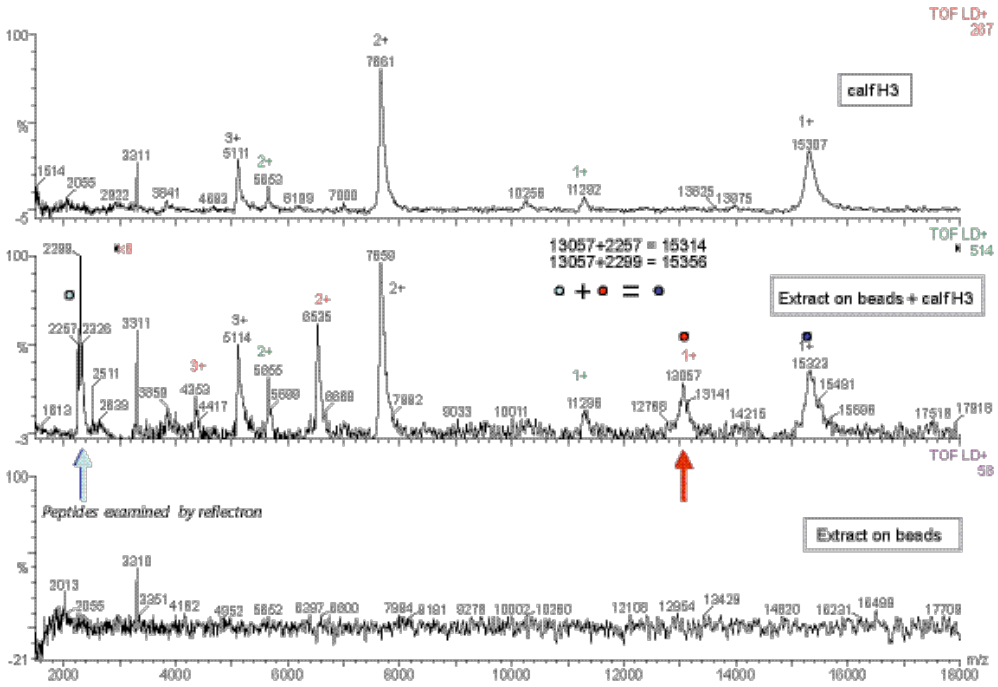
Supplementary Table 1

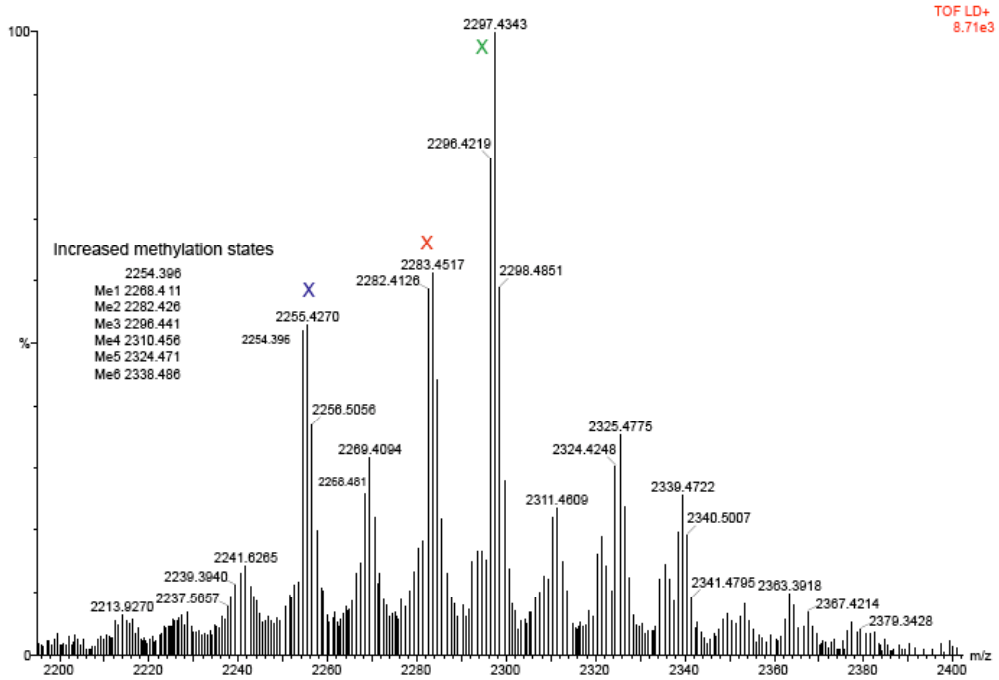
S1	NMA111
S8	PRB1
S8	YSP3
S8	KEX2
S8	YCR54C
S9	DAP2
S9	YNL320W
S10	YBR139W
S10	KEX1
S16	PIM1
S26	IMP1
S26	IMP2
S33	YJU3
S33	MET2
S33	ECM18
S33	ICT1
S54	YGR101W
S54	YOL107W
S52	RBD2
S59	NUP100
S9	STE13
S59	NUP145 (*unviable*)
S59	NSP116 (*unviable*)
S26	SEC11 (*unviable*)
S10	PRC1

S: Serine protease

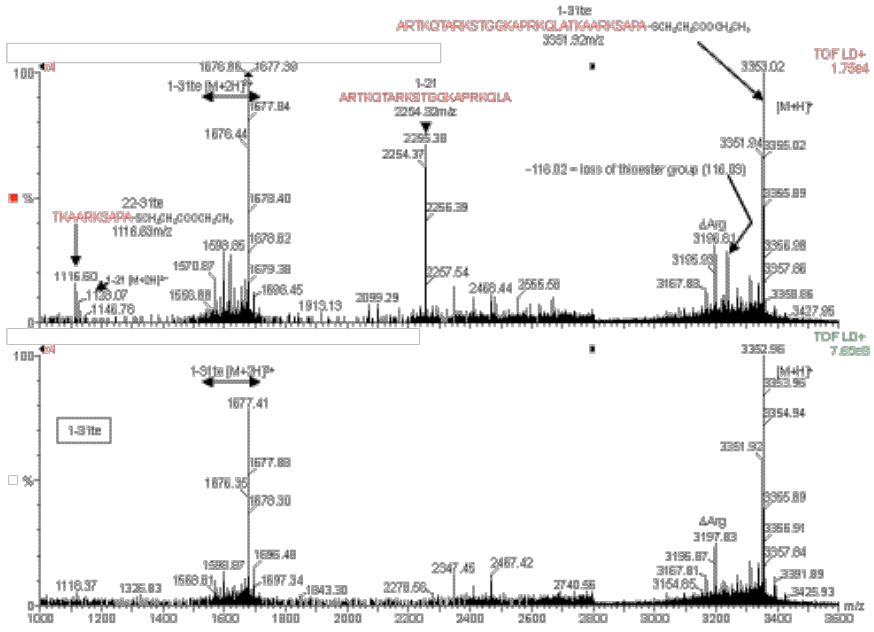
Table 1. Knocked out strains tested for H3 endopeptidase activity.
All strains were BY4741 background from Open Biosystem. Yeasts were grown to stationary phase (OD_{600mn}=5-7) and proteins bound to sepharose beads were assayed on recombinant histone H3. The activity was detected by western blot with anti H3 antibody.



S.1. Endopeptidase activity against calf histone H3.
Empty sepharose beads (upper panel) and extract from stationary phase (G0) on sepharose beads (middle panel) were assayed on thymus calf H3 (dark blue circle). Extract from stationary phase (G0) on sepharose beads was assyed in the absence of substrate as control (lowest panel). All reactions were analyzed by MALDI. The two peptides peaks product of the reaction are highlighted (red and light blue circle). The light blue peak was analyzed by reflectron (see S4).



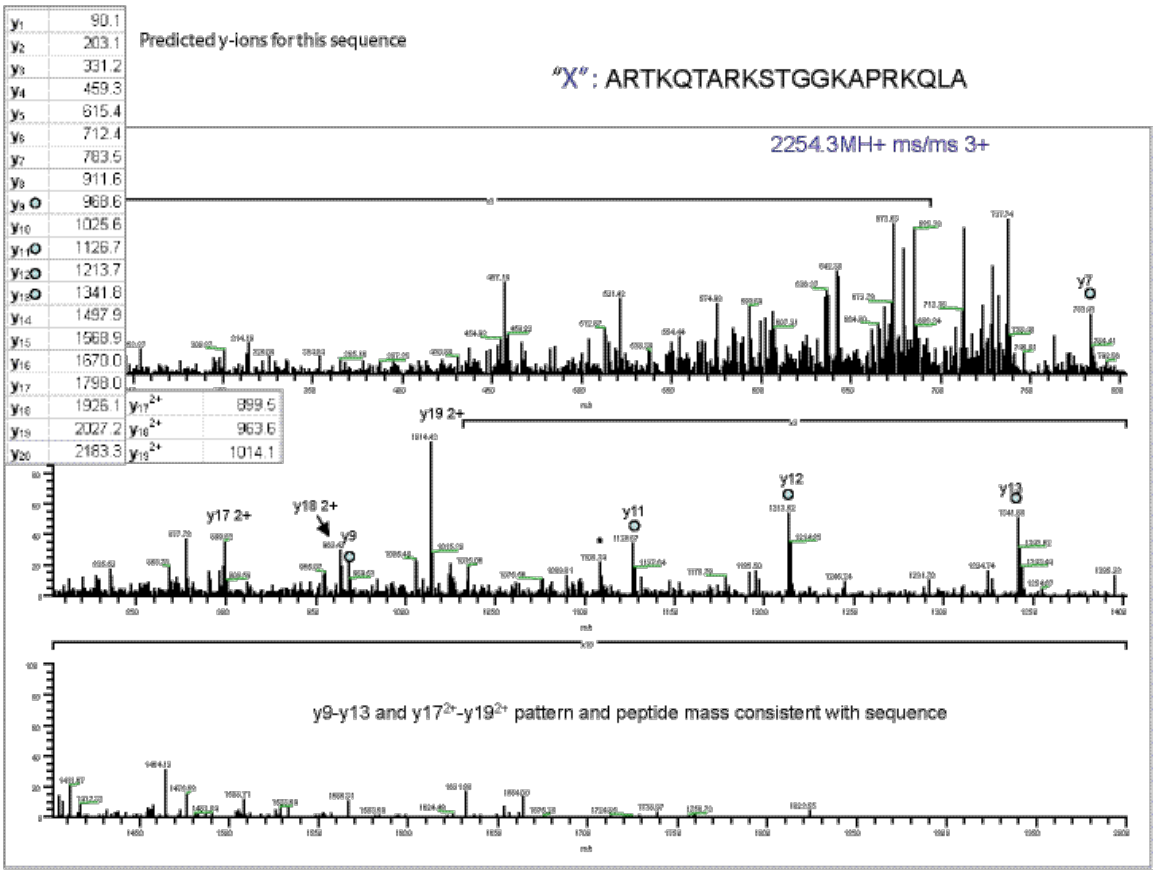
S. 2. Analysis by reflectron of the N-terminal product of calf H3 proteolysis.
aa2-aa22 = 2254.322. The idividual peaks correspond to combinations of methylation states on diferent residues of calf H3. Esi ms/ms was used to confirm the aminoacid sequence of the most prominet peaks marked with X (see S.4).



S.3. Endopeptidase activity against a synthetic H3 peptide.

Sepharose beads bound extract from yeast grown to stationary phase (G0) was assayed on peptide consisting of aa1-aa30 human H3 (upper panel). Empty sepharose beads were assayed on the same peptide as control (lower panel). Both reactions were analyzed by MALDI. The substrate and the two peptides product are highlighted (red).

1-31te = 1-31thioester



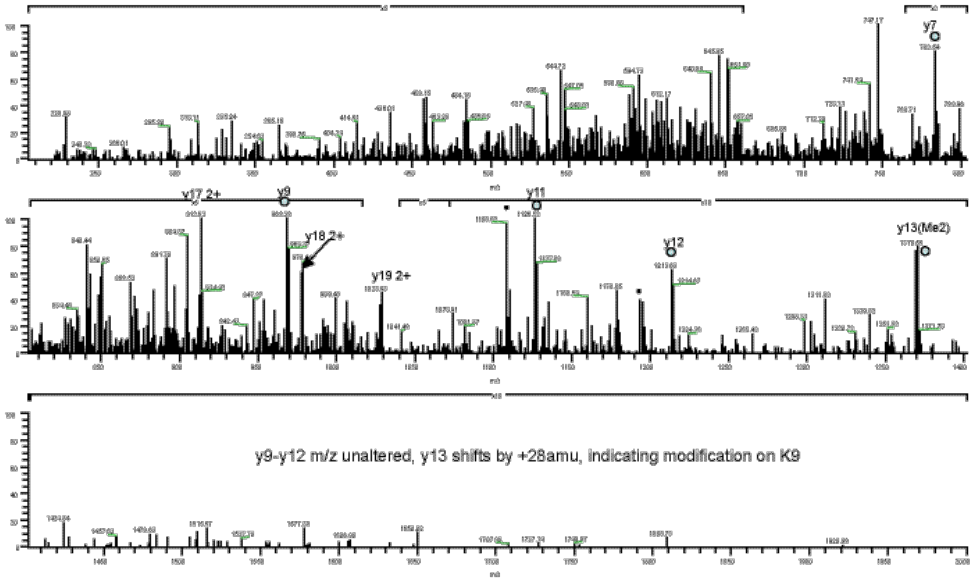
y_1	90.1
y_2	203.1
y_3	331.2
y_4	459.3
y_5	615.4
y_6	712.4
y_7	783.5
y_8	911.6
y_9 O	968.6
y_{10}	1025.6
y_{11}O	1126.7
y_{12}O	1213.7
y_{13}O	1341.8
y_{14}	1497.9
y_{15}	1568.9
y_{16}	1670.0
y_{17}	1798.0
y_{18}	1926.1
y_{19}	2027.2
y_{20}	2183.3

y_{17}^{2+}	899.5
y_{18}^{2+}	963.6
y_{19}^{2+}	1014.1

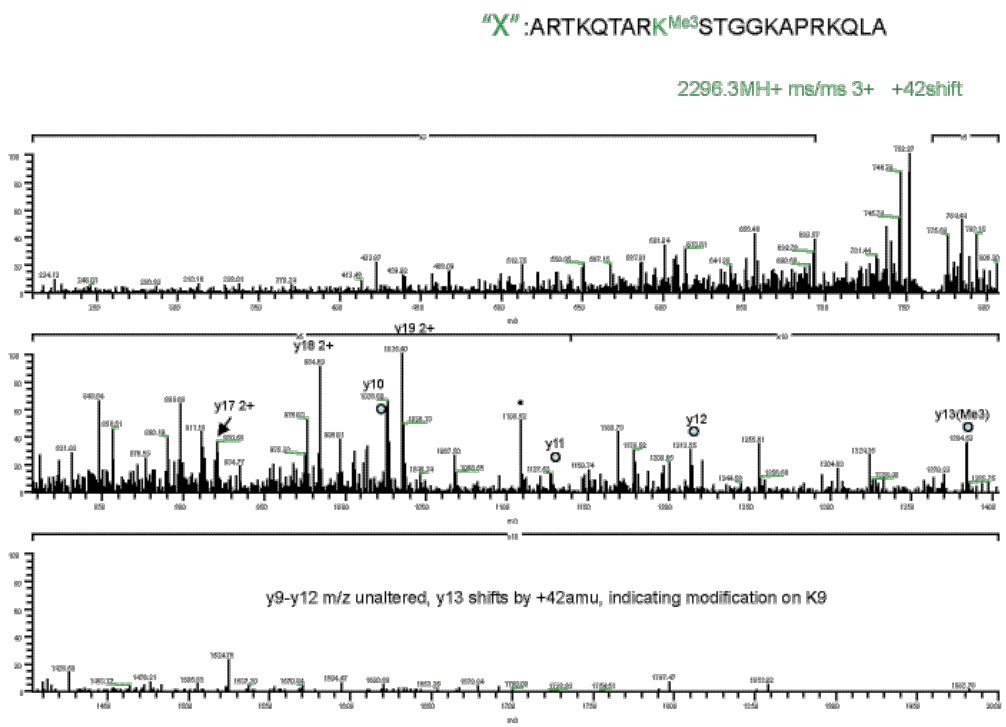
S 4. ES ms/ms analysis of the H3 endopeptidase N-terminal product "X".

"X": ARTKQTARK^{Me2}STGGKAPRKQLA

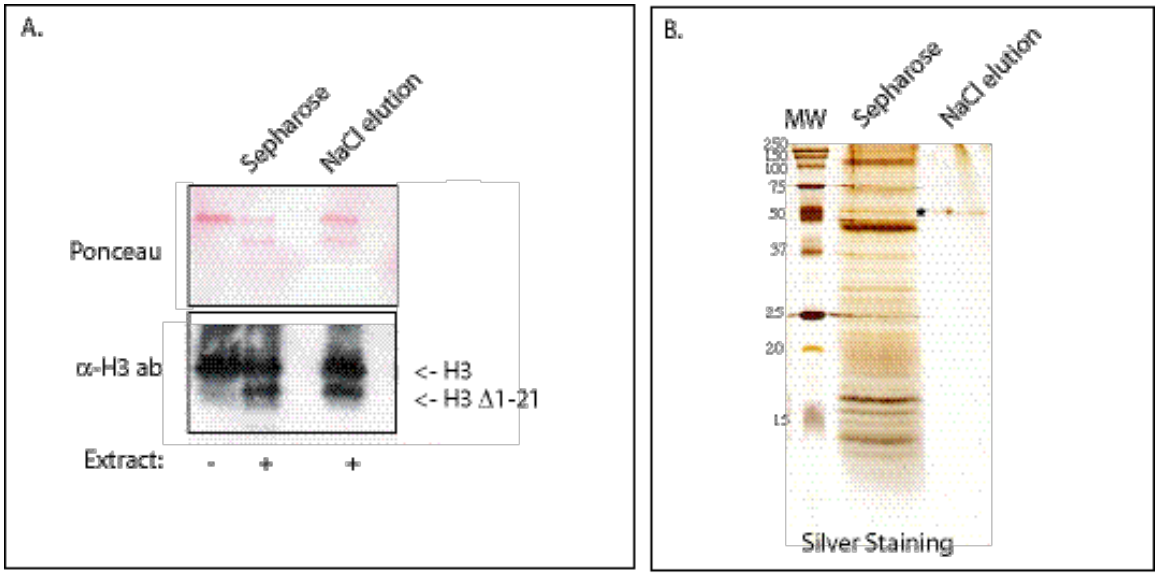
2282.4MH+ ms/ms 3+ +28shift



S 5. ES ms/ms analysis of the H3 endopeptidase N-terminal product "X".

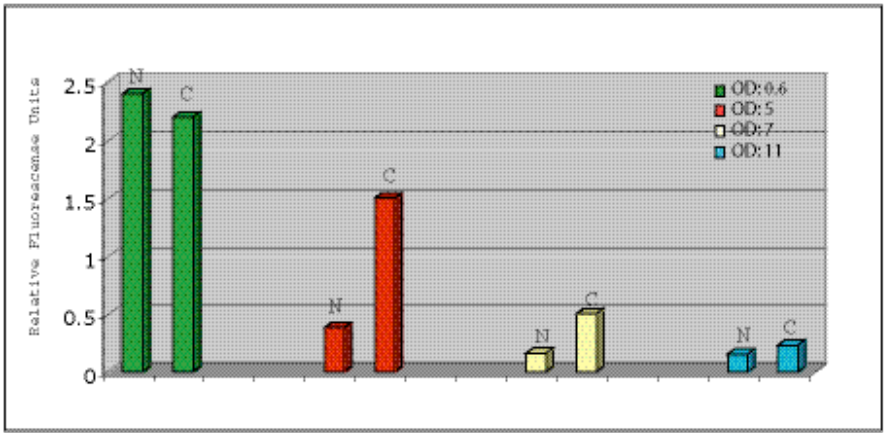


S 6. ES ms/ms analysis of the H3 endopeptidase N-terminal product "X".

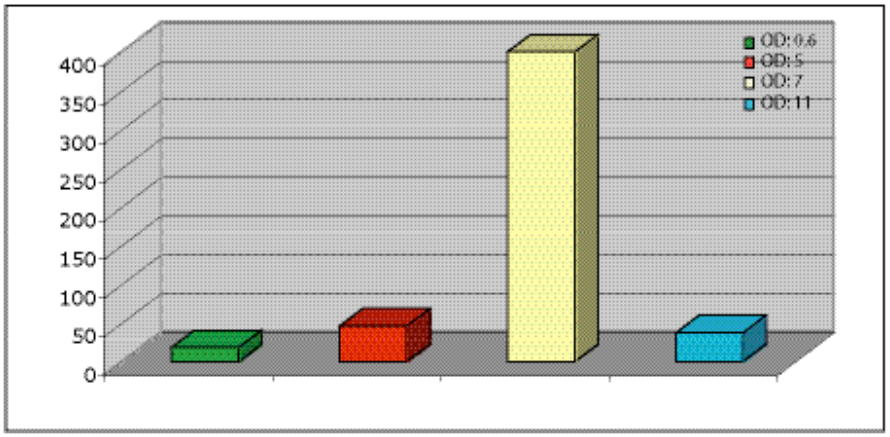


S7. Extract prepared from stationa y phase culture was pulled down on sepharose beads. Panel A: Bound proteins (Sepharose) and proteins eluted with 2M NaCL g (NaCl elution) were assayed for activity against calf histone H3. The reactions were analyzed by western blot with anti C-terminal H3 antibody. The H3 Clipped product is highlighted. Panel B: Silver staining of the fractions containing the endopeptidase activity. Sepharose: beads bound proteins. NaCl elution: 2M NaCL eluted proteins.

Chromatin Inmunoprecipitation HSP12 promoter



HSP12 mRNA level



S8. Chromatin immunoprecipitation experiments were performed in yeast cells cultured in glucose to OD600nm: 0.6 (green bars), OD600nm: 5 (read bars), OD600nm: 7 (yellow bars) and OD600nm: 11 (blue bars) using anti-myc antibody to detect the N-terminus of histone H3 (N) and anti H3 to detect the C-terminus of H3 (upper panel). The diagrams represent relative fluorescent units after normalizing the signal at the HSP12 promoter to an intergenic region on chromosome V. Lower panel: RT-PCR analysis was performed on the same cultures using primers specific to HSP12. The expression level of was normalized to the RNA levels of RTG2.